

Qualitative and quantitative evaluation of the ciliated protozoa in the middle northern coast of Egypt

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ABSTRACT

Protozoa play an important role in the different aquatic food webs. The main objective of this study is to identify the most abundant types of the ciliated protozoa at sampling stations extending along the middle northern coast of about 135 Km in Egypt. Three different classes of ciliated protozoa were detected at three sampling stations. These classes comprised seven subclasses which include 13 different orders. These orders were represented by more than 79 ciliate species. The numbers of the ciliate species at western Alexandria sampling station were 78, those of El-Hamaam achieved 70 and that of Sedi-A. Rhman reached 66 species only. The highest ciliate numerical densities were detected within class kinetofragminophora, followed by Oligohymenophora and then Polyhymenophora in the latter two stations, while those of Alexandria proved that Oligohymenophora exceeds the other two classes.

Data of the present study illustrated that the level of p^H is slightly alkaline (7.6-7.93), salinity is 8.2-12.3‰, water temperature is 11.3-27.4 °C, turbidity is 21.5-50.4 NTU, POC is 0.85 – 1.4 mg/L and dissolved oxygen is 6.3-7.8 mg/L. On the other hand, nutrient concentrations in that region of the Mediterranean sea are 2.9-7.4 µg/L for Ch-a and 0.21 - 0.61, 0.60 - 0.92, 0.019 -0.078 mg/l for silicate, total nitrates and total phosphates, respectively. Accordingly, it appears that these stations are more or less eutrophic particularly those of Alexandria followed by El-Hamaam and then Sedi-A. Rhman. Simultaneously, the nutrient concentrations of the present investigation are mostly higher as compared with those of the other studies. Comparing the numerical densities of both aquatic and bottom ciliated protozoan organisms belonging to the different classes at those three sampling stations proved that the ciliates inhabiting the sand grains of the latter are much higher than those of the former one by three to six times.

Key words: Ciliates, Mediterranean sea, Northern coast.

INTRODUCTION

Protozoa are important biotic components in the aquatic ecosystem, particularly ciliates, which act as predators of bacteria and other micro-organisms beside some rotifers. In addition, they provide nutrition for organisms at higher trophic levels (Kneitel & Chase, 2004 and Dopheide *et al.*, 2009), increase mineralization and make nutrients more available to other organisms (Vickerman, 1992). Protozoa play also a crucial role in the food chains as bio-monitors and/or indicators of water quality (Charubhun and Charubhun, 2000). Few protozoan data have been collected in several zooplankton studies along the Egyptian Mediterranean Coast (Abdel-Aziz, 2004, Abdel-Aziz & Aboul-Ezz, 2003; Anon, 2007). Marine protozoa comprise certain groups such as radiolarians and foraminiferans that have biological significance in sediments only. Other marine sarcodines can not be identified due to their body fragility and their relatively low quantitative and qualitative values. Most of the flagellates are difficult to be examined microscopically according to their very minute size. On the other hand, most of marine free-living ciliates are widely distributed, dominate in both species numbers and numerical densities. In addition, protozoan organisms play a vital role

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in the microbial food web (Lee & Choi, 2000) beside their high tolerance against extreme environmental conditions such as heavy metals and sewage pollution (Forge *et al.*, 1993).

Diversity and structure of ciliate communities in Gabes' Gulf in Tunisia were investigated at near-shore stations along 237 Km. where high ciliate species richness was recorded (Elloump *et al.*, 2015).

Ciliated protozoa as microbivores appear to stimulate the rates of carbon and nitrogen cycling (Fenchel, 1987; Finlay *et al.*, 2004). Great numbers of ciliates feed mostly on bacteria and not on dissolved organic material and compete with other ciliates and rotifers for different bacteria. Damietta coast as a part of the Egyptian Mediterranean coast is considered as a polluted area due to an exaggerated human activity. The environmental parameters of previous investigations showed low salinity, mild anoxic conditions, enough nutrient levels and quite phytoplankton growth. A total of 69 protozoan species were identified and their annual numerical densities varied between 8.2×10^3 and 51.4×10^3 cells m^{-3} and the highest values were obtained during Spring (Dorgham *et al.*, 2013).

The population densities of filter-feeding ciliates at three marine protectorates (Ras Mohammed, Nabq and Abu Galoum) in Al-Aqaba Gulf at the northern Red Sea, were evaluated by El-Serehy *et al.* (2012). The ciliate abundance in these sampling sites were found to vary according to an annual cycle where the highest ciliate numerical densities (2.5×10^4 cells/ L) was obtained on spring and the lowest values (0.2×10^4 cells/ L) was detected during summer.

MATERIALS AND METHODS

The studying area extends along the Middle Northern Mediterranean coast of Egypt for about 135 KM. It includes three sampling stations; western coast of Alexandria city, El-Hamaam and Sedi A. Rahman towns as could be seen in Figure (1).

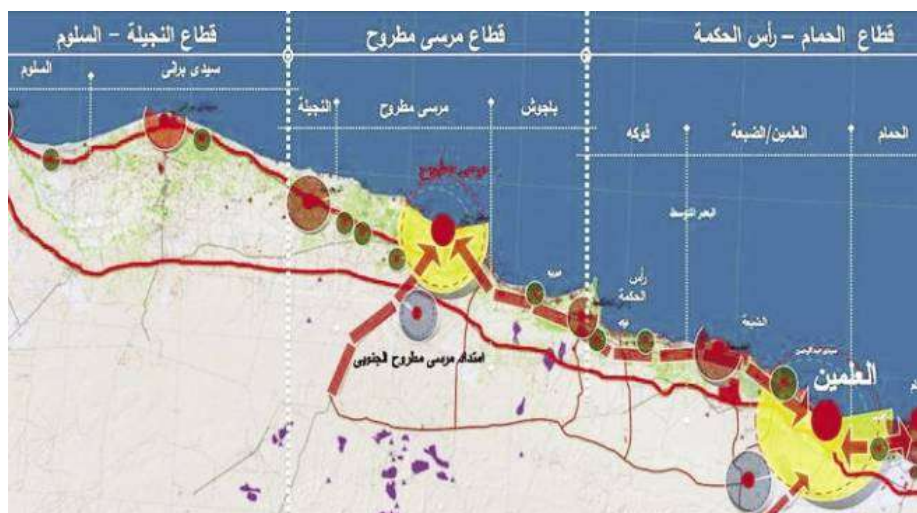


Fig. (1). Map of sampling sites at the Middle Northern Mediterranean coast of Egypt ; western coast of Alexandria city, El-Hamaam and Sedi A. Rahman towns (Source : Google Earth).

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Sampling was carried out during March (1, 8, 15, 22, 29) and April (5, 12, 19, 26), 2014. A total of 27 sediment samples and another 27 aquatic ones were collected from those sampling stations. Sand samples were picked up early morning at about 50 cm water depth by pressing a rectangular brass corer of 10 cm length and 1 cm² cross area into the sandy bottom to collect sand and its protozoa, Sand of each corer was placed in a glass funnel coated internally with a double- muslin layer in order to extract protozoa and to prevent sand grains to pass. Each funnel was placed over a 100 ml- cylindrical Perspex chamber (Fig. 2).

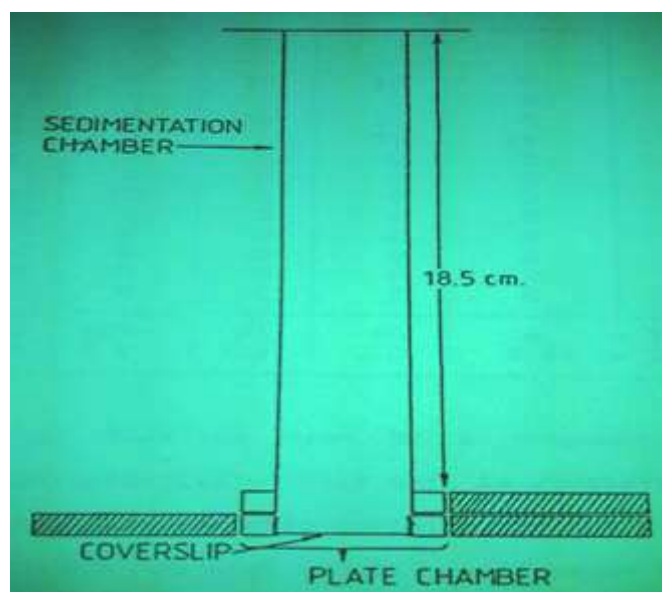


Fig. (2). The Sedimentation Chamber for ciliated protozoa

This was followed by pipetting sea water above the sand samples to drive the protozoan organisms away (Galal,1989) . On the other hand, sea water was collected using a perspex sampler (of one-liter volume) at about 30 cm below water surface at the same sampling sites. Enumeration, identification and classification of these protozoans were carried out via Corliss (1979) and Levin *et al.* (1980). Microscopical examination was carried out by the help of Carl- Zeiss Jena transmitted- light inverted microscope. It was found helpful to slow down the rapid movements of many of these ciliated organisms by adding few drops of methyl cellulose solution (15 gm in 85 ml water) to the preparation inside the basal part of the sedimentation chamber. Most of the physico-chemical parameters of water such as salinity, POC, turbidity, dissolved oxygen, pH, Ch-a, silicates, total phosphate and nitrate salts were measured depending on APHA (2005).

RESULTS

The microscopical examination revealed that different organisms belonging to many taxa were present in the collected samples from the three sampling stations at the middle part of the egyptian northern coast. These organisms include rotifers, worms (nematodes and polychaetes), crustacean larvae and tremendous types and numbers of different protozoan organisms. Protozoa were proved to be the most abundant group among other organisms. They are represented by two phyla; Sarcomstigophora and Ciliophora.

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Ciliated protozoan organisms were proved to be higher than both sarcodines and mastigophoreans from diversity and numerical density point of view. Subphylum Sarcodina is represented, in the present study, by Rhizopodea (naked amoebae) such as *Arcella discoids*, *A. vulgaris*, *Amoeba proteus* & *A. striata* and Actinopodea (testate amoebae) comprising *Actinosphaerium* sp., *Centropyxis* sp., while Mastigophora comprises Phytomastigophorea (*Oicomonas*, *Chilomonas*, *Cryptomonas*, *Euglena*, *Peranema* and *Heteronema* species) and Zoomastigophorea (*Monosiga* sp and *Bodo* sp.). On the other hand, phylum Ciliophora is represented totally in those three sampling stations by more than 79 ciliate species/genera belonging to 13 orders of three main classes, namely Kinetofragminophora, Oligohymenophora and Polyhymenophora . It was found that the number of the ciliate species (species richness) at Alexandria sampling station is 78, that of El-Hamaam is 70 and that of Sedi-A. Rhman is 66 individuals of this protozoan class (Ciliophora).

Data in Table (1) indicated that Alexandria sampling station has higher numerical densities of ciliated protozoa than those of El-Hamaam then Sedi- A. Rhman. The highest ciliate numerical densities were found in class kinetofragminophora followed by those of Oligohymenophora and then those of Polyhymenophora at the latter two sampling stations. At Alexandria station those of Oligohymenophora exceeds those of the other two classes (Kinetofragminophora and Polyhymenophora). Simultaneously, class kinetofragminophora exhibited 40 different genus/species ciliate organisms whose numerical densities are 1680, 1130 & 984/L at Alexandria, El-Hamaam and Sedi-A. Rahman, while Oligohymenophora was represented by 23 types of various protozoan species and the densities are 2028, 813 & 688/L, respectively.

Table (1). Numerical densities (n° /L) of ciliated Protozoa belonging to different classes in the middle northern coast of Egypt.

Protozoan organisms	Alexandria	El-Hamaam	Sedi-A. Rhman
Class1 Kinetoframinophorea	1680	1130	984
S.class1 Gymnostomatia	(35.8%)	(44.5%)	(44.3%)
Order: Karyorelictida <i>Loxodes magnus</i>			
O: Prostomatida <i>Holophrya ehrenbeg</i> <i>Vasicola tatem</i> <i>Urotricha</i> sp <i>Coleps</i> sp <i>Prorodon</i> sp			
O: Haptorida <i>Lacrymaria olor</i> <i>Homalozoon</i> sp <i>Spathidium</i> sp <i>Dileptus anser</i> <i>Askenasia</i> sp. <i>Mesodinium rubrum</i>			
<i>M. pulex</i> <i>Monodinium</i> sp. <i>Didinium</i> sp <i>Trachelius ovum</i> .			
O: Pleurostomatida <i>Acinaria incurvata</i> <i>Amphileptus lanceolus</i> <i>Litonotus lamella</i> <i>L. fasciola</i> <i>L. Anguilla</i> <i>L. dusarti</i> <i>L. Cygnus</i> <i>Loxophyllum asetosum</i> <i>L. dragescoi</i> <i>L. kah</i> <i>L. laevigatum</i> <i>L. multiplicata</i> <i>L. niemeccense</i> <i>L. verrucosum</i> <i>Hemiophrys bivaculata</i> <i>H. pleurosigma</i>			
S.class2 Hypostomatia			
Order: Cyrtophorida <i>Chilodonella uncinata</i>			
S.class3 Vestibulifera			
Order: Colpodida <i>Colpoda steini</i> <i>C. cucullus</i>			
Order: Trichostomatida <i>Plagiopyla</i> sp.			
S.class4 Suctoria	2028	813	688
Order: Suctorida	(43.2%)	(32.0%)	(31.0%)
<i>Acineta tuberosa</i> <i>A. infundibuliformis</i> <i>Podophrya fixa</i> <i>Tokophrya</i> sp.			
Class2 Oligohymenophorea			
S.class1 : Hymenostomatia			
Order: Hymenostomatida <i>Uronema nigricans</i> <i>U. filificum</i> <i>Cyclidium caudatum</i> <i>C. glaucoma</i> <i>C. heptatrichum</i>			
<i>Cinetochilum margaritaceum</i> <i>Paramecium caudatum</i> <i>P. putrinum</i> <i>Paramecium virginianum</i> <i>Colpidium campylum</i>			
<i>C. colpoda</i> <i>Frontonia marina</i>	987	599	548
S.class2 : Peritrichia	(21.0%)	(23.5%)	(24.7)
Order: Peritrichida <i>Vorticella campanula</i> <i>V. striata</i> <i>V. convallaria</i> <i>V. microstomum</i> <i>Epistylis</i> sp			
<i>Charchesium</i> sp. <i>Trichadina pediculus</i> <i>Cothurina</i> sp <i>Opercularia coarctata</i> <i>O. nutans</i> <i>Hastatella</i> sp			
Class3 Polyhymenophorea Subclass1 : Spirotrichia			
Order: Heterotrichida <i>Stentor coeruleus</i> <i>S. roeseli</i> <i>Condylostoma</i> sp. <i>Metopus</i> sp. <i>Spirostomum Ehrenberg</i> .			
Order: Hypotrichida <i>Aspidisca ehrenberg</i> <i>A. castata</i> <i>A. ciccada</i> <i>Urostyla</i> sp. <i>Stylonychia mytilus</i> <i>S. putrina</i> .			
<i>Oxytricha falla</i> <i>Tachysoma pellionella</i>			
Order: Oligotrichida <i>Codonella</i> sp. <i>Tintinnopsis</i> sp. <i>Halteria</i> sp.			

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On the other hand, Polyhymenophora was illustrated by 16 ciliated protozoan genus/species whose densities are 987, 599 & 548/L. At the same time, the percentages of the three ciliate classes at both El-Hamaam and Sedi-A.Rhman are more or less similar, 44.5% and 44.3%, for Kinetofragminophora, 32.0% and 31.0% for Oligohymenophora, 23.5% and 24.7% for Polyhymenophora, while those of Alexandria sampling station were lesser than those of the previously mentioned stations apart from that of class Oligohymenophora.

Regarding the ciliate numerical densities and their percentages belonging to the different orders, it was proved that the ciliate orders having the highest numerical densities are restricted to Alexandria station followed by those of Sedi A. Rhman. On the contrary, the highest percentages of these orders are belonging to Sedi A. Rhman followed by those of western Alexandria and at El-Hamaam as could be seen in Table (2).

Table (2). Numerical densities (n^o/L) of ciliates belonging to different orders and their percentages at various sampling stations in the middle northern coast of Egypt.

Protozoan orders	Alexandria		El-Hamaam		Sedi- A. Rhman	
	N ^o	%age	N ^o	%age	N ^o	%age
Class1 Kinetofragminophoera						
Order: Karyorelictida	33	0.7%	19	0.8%	54	2.4
O: Prostomatida	197	4.0	30	1.2	72	3.2
O: Haptorida	508	10.8	362	14.2	214	9.6
O: Pleurostomatida	466	9.9	301	11.8	201	9.1
Order: Cyrtophorida	299	6.4	250	9.8	274	12.4
Order: Colpodida	67	1.4	78	3.1	79	3.6
Order: Trichostomatida	30	0.6	36	1.4	42	1.9
Order: Suctorida	90	1.9	54	2.1	48	2.2
Class2 Oligohymenophorea						
Order: Hymenostomatida	1050	22.4	521	20.0	389	17.5
Order: Peritrichida	978	20.8	292	11.5	299	13.5
Class3 Polyhymenophorea						
Order: Heterotrichida	123	2.6	49	1.9	150	6.8
Order: Hypotrichida	814	17.3	488	19.2	308	13.9
Order: Oligotrichida	50	1.1	62	2.4	90	4.1

Comparing the numerical densities of both aquatic and sediment ciliated protozoan organisms belonging to the previously mentioned classes at the various sampling stations indicated that the sediment samples having higher ciliate numerical densities than those of the aquatic ones by more or less three to six times as could be predicted from Table (3).

Sampling station	Alexandria		El-Hammam		Sedi.A. Rhman	
	S	A	S	A	S	A
Class: Kinetofragminophorea	1680	389	1130	282	984	184
Class: Oligohymenophorea	2028	512	813	178	688	148
Class: Polyhymenophorea	987	361	599	126	54	114
Total	4695	1262	2542	586	2220	446

Table (3). Aquatic (A) and sediment (S) numerical ciliate densities at various sampling stations in the middle northern coast of Egypt.

The present study illustrated that the values of p^H are slightly alkaline (7.6-7.93), the salinity is more or less 32 - 39‰, water temperature is 11.3-27.4 °C, turbidity is 21.5-50.4 NTU and dissolved oxygen is 6.3-7.7 mg/L, while those of particulate organic carbon (POC) ranged between 0.85 and 1.4 mg/l. On the other hand, nutrient concentrations along that

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region of the Mediterranean sea for Ch-a is 2.9-7.4 ug/L, while those of silicate, total nitrates and total phosphates are 0.021 -0.061, 0.060-0.092, 0.019-0.078 mg/l respectively (Table 4). Simultaneously, the influence of certain physico-chemical parameters on the numerical densities of both aquatic and sediment ciliated protozoa was examined statistically to detect the importance of these factors on the distribution and the abundance of these unicellular organisms.

Table (4). Values of certain environmental parameters at three sampling stations in the middle northern coast of Egypt.

Stations	Alexandria	El-Hamaam	Sedi-A.ERhman
Temperature [°C]	11.3 – 25	10.5 – 26	11.8 – 27.4
Salinity (‰)	32.5	35.9	39.1
Turbidity (NTU)	50.4	31.0	14.9
P ^H	7.6	7.81	7.93
Dis. Ox. (mg/L)	6.3	7.4	7.7
POC (mg/L)	1.4	1.1	0.85
Total NO ₃ (mg/l)	0.096	0.072	0.060
Total PO ₄ (mg/l)	0.078	0.034	0.019
Ch-a (ug/l)	2.9	3.6	4.7
Silicate (mg/l)	0.021	0.049	0.061

DISCUSSION

In aquatic environments, ciliated protozoan assemblages are important components of the microplankton fauna and are considered primary mediators of energy transfer from pico- and nanoplankton production to higher trophic levels in the functioning microbial loop (Dolan and Coats 1990; Sime-Ngando *et al.* 1995; Jiang *et al.*, 2013). It is becoming increasingly recognized that there are several advantages in using ciliated protozoa for the assessment of water quality. As a result of their short life cycles and semipermeable external membranes, they may react more rapidly to the environmental changes than many other eukaryotic organisms. Furthermore, many forms can inhabit environments that are unsuitable to metazoan organisms (Cairns *et al.*, 1972; Franco *et al.*, 1998; Corliss 2002; Madoni and Braghiroli 2007; Jiang *et al.*, 2007).

Parallel to the data obtained by Dorgham *et al.* (2013), human activities along a coastal region have caused drastic changes in the environment, expressed by a salinity decrease, frequent low and slightly moderate oxygenated conditions and elevated nutrient levels. These changes are reflected in the structure and abundance of the protozoan community in the studying area. From the nutrient concentration point of view, the present sampling stations could be classified as eutrophic with particular reference to those of the former one (west Alexandria station) which is more or less similar to those obtained by Vucak & Stirn (1982), Ignatiades *et al.* (1992) and Marchetti (1984).

On the other hand, the importance of functional groups of ciliates from marine ecosystems has not been well recognized, especially with respect to the potential capability in the assessment of water quality (Jiang *et al.*, 2013). Jiang *et al.* (2011) revealed that spatio-temporal patterns of ciliate communities are significantly correlated with environmental conditions and that some dominant species are significantly correlated with concentrations of nutrients. Thus, it is possible to conclude that some ciliate assemblages, as functional groups with specific role in communities, could respond predictably to different environmental conditions.

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According to Balkis (2004), Bel Hassen *et al.* (2008), Drira *et al.* (2010) and Hannachi *et al.* (2009), the quantitative dominance of oligotrich ciliates was found to be related to the oligotrophic status of the studying area at Gulf of Gabes. Simultaneously, it was proved that the quantitative dominance of certain tintinnid ciliated protozoan organisms was related to the pollution level at the same gulf in Tunisia (Drira *et al.*, 2008; Hannachi *et al.*, 2009). The differences in the community composition of tintinnid ciliates suggest that ciliated protozoan communities in the east and central Mediterranean are more diverse in terms of numbers of species, and species evenness (Dolan *et al.*, 1999). It was proved (Elloumi *et al.*, 2015) that class *Spirotrichea* was the most abundant ciliates at all the examined stations where they represented about 36.6 % of the total abundance. The class of Spirotrich ciliates was followed by the oligohymenophoreans, with the highest percentage (30 %) of the total abundance recorded and then Heterotricheas are contributed to 10 % and 50 % of total abundance and biomass. Karyolectea and Colpodea were the least abundant classes; they did not exceed 10 % of the total ciliate abundance and biomass.

Simultaneously, Dorgham *et al.* (2013), emphasized that *Bursaridium* sp., *Frontonia atra*, *Holophrya* sp., *Paramecium* sp., *Vasicola ciliata*, and *Vorticella* sp. can be considered as indicators of eutrophication. *Vorticella* sp. stands out for its tolerance to a highly polluted environment exactly as proved by Salvado *et al.* (1995). In addition, *V. ciliata* was recorded in a stressed area at west of Alexandria coast (Abdel-Aziz, 2005).

The high abundance of ciliates indicated mostly that organic and nutrient materials might pollute the water bodies. However, neither species numbers nor abundance can be individually used for the bioassessment of water conditions. Number of species is sometimes not sensitive enough to reflect minor changes of water conditions. In addition, number of species may be more or less influenced by personal observations. On the other hand, sampling periods and intervals, geographical sites beside the tidal effects may reduce the statistical analysis consistency (Dolan and Coats, 1990; Park and Choi, 1997). A total of 8,000 described ciliate morphospecies include about 200 fossil tintinnids and 2,600 as commensals, leaving about 5,200 free-living species (Corliss, 2000). Regarding the relationships between the ciliated protozoan numerical densities and some of the physico-chemical properties from the statistical point of view, it was proved that ciliates of the water samples are statistically significant in case of water temperature ($P=0.026$), dissolved oxygen ($P=0.03$) and total phosphates ($P=0.03$), while those of sand samples are significant with dissolved oxygen ($P=0.05$) and total phosphates only ($P=0.02$).

Making a comparison between data of the physico-chemical parameters of the present study with those of Emam *et al.* (2013), it was found that salinity levels achieved 32-39‰ in the first study, 33‰ For the second. The p^H values proved to be slightly alkaline (7.6-7.98) at the previous studies, Those of dissolved oxygen were (6.3-7.7) and (6.9) mg/L respectively, while silicate concentrations were found to be (0.02-0.06) and (1.035) mg/L respectively. On the other hand, the nutritional levels of nitrates and phosphates for these two studies proved that nitrates are represented by (0.06-0.096) and (0.037) mg/L, while those belonging to total phosphates are (0.02-0.08) and (0.08) mg/L, respectively.

The present study indicates that the average total ciliate densities were 4695, 2542 and 2220/L at western Alexandria, El-Hammam and Sedi A. Rhman respectively which means that ciliate numerical densities increased in the direction of east-west of the Mediterranean sea which is more or less similar with the results obtained by Pitta *et al.* (2001). At the same time, the higher numerical densities of ciliated protozoa of the sandy bottom samples, as compared with those of water samples belonging to those near-shore collections, could be referred to availability of the nutritive requirements and weak currents in the former

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sampling stations. Variations in the present data could be attributed mostly to the illegal sewage discharge at the adjacent of Alexandria sampling station and/or due to predation influence of certain animals such as crustaceans, insect larvae and rotifers (Dolan *et al.*, 1999 and Pitta *et al.*, 2001). This conclusion is confirmed by data of the particulate organic carbon, total nitrates and phosphates. It was proved that ciliates of the water samples are statistically significant with water temperature ($P=0.026$), dissolved oxygen ($P=0.03$) and total phosphates ($P=0.03$), while those of sand samples are significant only with dissolved oxygen ($P=0.05$) and total phosphates only ($P=0.02$).

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التقدير النوعي والكمي للكائنات الهدبية الأولية في الساحل الشمالي الأوسط لمصر.

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المستخلص

تلعب الكائنات الأولية دوراً هاماً في السلاسل الغذائية المائية بمختلف أنواعها. لذلك أجريت هذه الدراسة للتعرف على التوزيع البيئي لتلك الكائنات الأولية خاصة الهدبية منها بثلاث مناطق مختلفة (غرب الاسكندرية والحمام وسيدي عبد الرحمن) تمتد لمسافة مائة وخمس وثلاثون كيلومتر تقريباً على الجزء الأوسط من الساحل الشمالي بجمهورية مصر العربية. ولقد وجدت انواع مختلفة من تلك الكائنات الأولية بلغت اكثر من تسع وسبعون جنساً ونوعاً تتبع طوائف ورتب وعائلات مختلفة. ولقد اتضح من هذه الدراسة أن توزيع تلك الكائنات يختلف من منطقة لأخرى استناداً الى الاختلاف في نوعية وكمية المواد الغذائية بالإضافة الى بعض العوامل البيئية المختلفة كالعوامل الطبيعية والكيميائية مثل الأس الهيدروجيني والملوحة ودرجة حرارة الماء والعاكسة والأكسجين الذائب في الماء. هذا بالإضافة الى بعض المواد الغذائية الضرورية مثل الكربون العضوي والنترات والفوسفات ولكن بدرجات متفاوتة استناداً الى التحاليل الاحصائية. ويتضح بذلك ان تلك المناطق التي تمت فيها هذه الدراسة غنية بالمواد والعناصر الضرورية لاغتذاء الأسماك والكائنات البحرية ذات القيمة الغذائية والاقتصادية. كما اثبتت هذه الدراسة ايضاً ان تلك الكائنات تلعب دوراً هاماً في ازالة بعض اسباب التلوث في هذه المناطق البحرية. ولقد اتضح ان الكثافة العددية لتلك الهدبيات الأولية تكون أكبر في طبقة الرمال حتى عمق 10 سم عنها في عمود الماء الذي يعلوها بثلاث الى ست مرات.